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APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

REMARKS

Applicants note that the Examiner has withdrawn the previous rejections under 35 U.S.C. §112, 1st paragraph (enablement). The sole remaining rejections in this case are maintained novelty and obviousness rejections (of all claims except claim 53) are based entirely on the same art (Kanner, Wirth, Pidgeon, and Stoughton) previously cited by the Examiner, as distinguished at length by Applicants in their previous Response. The Examiner has held claim 53 allowable if rewritten in independent form including all limitations of claim 1, from which it depends.

The present Response is filed subsequent to a telephonic interview with the Examiner, conducted on December 15, 2006, in which certain claim amendments (presented herein) and the Examiner's continued concerns about the teachings of the Kanner and Wirth references were discussed. Independent claims 1 and 30 have been voluntarily amended. Upon entry of this amendment, claims 1-39 and 49-53 are presently pending and under consideration (claims 40-48 are withdrawn).

Independent claims 1 and 30 have been voluntarily amended to more distinctly point out the characteristics and features of the claimed subject matter, as suggested by the Examiner. More particularly, the preamble of claims 1 and 30 have been amended to clarify that the method of the invention pertains to isolating a "target" population of modified peptides from a complex mixture of peptides. Similarly, step (c) of these independent claims have been amended, for consistency with the preamble, to recite that "said target" population of modified peptides is isolated.

These amendments are supported throughout the specification and claims as originally filed, for example, at p. 28, lines 12-22, p. 42, lines 25-30, and the Examples. The present amendments do not introduce new matter.

SUMMARY OF TELEPHONIC INTERVIEW

Applicants thank the Examiner for the courtesy of the telephonic interview (the third interview conducted in this case) of December 15, 2006. Also participating in the interview were Supervisory Patent Examiner Long Le (Art Group 1641) and Dr. John Rush, Ph.D., the first named inventor in this case and a person of skill in the art to which the invention pertains.

During the interview, Applicants' attorney discussed with the Examiner two amendments to the claims that the Examiner had previously suggested in a telephone call on November 24, 2006.

Firstly, it was suggested that Applicants insert the word "target" before "population" in the preamble of independent claims 1 and 30, and similarly replace the phrase "at least one population" in step (c) and step (d), respectively, of these claims with "said target population." Secondly, it was suggested that Applicants replace the phrase "comprises a complex mixture of peptides" in step (a) of these two independent claims with "consists of a complex mixture of peptides." Applicants' attorney indicated that the first suggested amendment was agreeable and would be made upon filing of this Response (this amendment is the only amendment presently made to the claims in this paper). Applicants' attorney informed the Examiner that the second suggested amendment would not be made, as it would unduly and unfairly limit the scope of the subject matter to which Applicants are entitled.

Particularly, Applicants' attorney informed the Examiner that the proposed "consisting of" limitation is inappropriate because the complex mixture of peptides required in Applicants' method is not limited to a mixture that consists of *only* peptides. Quite to the contrary, the Examiner was informed that a central feature of Applicants' invention is the ability to isolate a desired target population of post-translationally modified peptides from a complex mixture that contains a diverse range of different small peptides (both modified and unmodified) but may well also contain other non-peptide cellular constituents, such as whole proteins, nucleic acids, and cell membrane carbohydrates. The Examiner was reminded that this central feature is not taught, nor enabled, in any way by Kanner or Wirth. *See* Remarks below, for further discussion.

Specifically discussed were the Examiner's contentions that Kanner and Wirth *inherently* isolate a population of desired peptides from a complex peptide mixture. Applicants' attorney drew the Examiner's attention to the fact that the cited references both teach the use of proteolytic inhibitors in order to prevent peptide formation and maintain mostly whole protein in their samples, in contrast to the invention's use of a complex mixture of largely peptides derived from whole proteins. The Examiner's attention was also drawn to the fact that the cited references both fail to isolate any peptides because they teach the use of a gel separation step in which any peptide would in fact likely be lost, in contrast to the invention which isolates and captures desired peptides. *See* Remarks below for further discussion.

At the conclusion of the interview, Applicants' attorney directly asked the Examiner and her SPE if either had any remaining concerns about the patentability of the invention over the cited references that had not be resolved in the Interview. Both Examiners indicated they did not. Applicants' attorney stated that if no other concerns remained, then Applicants' would expect an allowance to issue in the case upon filing of their Response. Both Examiners indicated that no further

concerns or issues remained which would preclude a notice of allowance from issuing.

Applicants' attorney concluded the telephonic interview by informing the Examiner that Applicants' would file this Response entering the first suggested amendment to the independent claims, which merely clarifies that the population of modified peptides isolated is the one targeted for isolation from a complex mixture of peptides.

NOVELTY REJECTIONS (MAINTAINED)-- BASED ON KANNER AND WIRTH

The Examiner has maintained the rejection of claims 1, 10, 13-16, and 19 under 35 U.S.C. §102(b) as allegedly being anticipated by Kanner *et al.*, *J. Immunol. Meth. 120:* 115-124 (1989) (hereinafter "Kanner")). The Examiner continues to assert that Kanner disclose the selective immunoaffinity isolation of a population of post-translationally modified peptides from complex mixtures of peptides using phospho-tyrosine-specific antibodies, and thus anticipates the claimed invention (*see* November 20, 2006 Office Action at p. 3).

The Examiner has also maintained the rejection of claims 1, 2, 4, 5, 10, and 13-23 under 35 U.S.C. §102(b) as allegedly being anticipated by Wirth *et al.*, *Electrophoresis 14:* 1199-1215 (1993) (hereinafter "Wirth")). The Examiner continues to assert that Wirth disclose the selective immunoaffinity isolation of a population of post-translationally modified peptides from complex mixtures of peptides using phospho-tyrosine-specific antibodies, and thus anticipates the claimed invention (*see* November 20, 2006 Office Action at p. 4-5).

More specifically, the Examiner bases the maintained rejections on the following contentions (*see* page 14-15 of the November 20, 2006 Office Action):

- that "it is inherent that that there is a complex mixture of peptides [as taught by the present invention] present in the . . . preparation of Kanner and Wirth" (Id. at p. 14, first para., lines, 4-5);
- that "Wirth and Kanner are also able to bind to peptides possibly present in the proteinaceous preparation in the same manner as that of the present invention" (Id. at p. 14, first para., lines 12-13);
- that "The open 'comprising' language [as recited in claims 1 and 30] has other molecules, such as proteins, other than peptides"(Id. at p. 14, first para., lines 8-9); and,
- that " 'selectively isolating one population of peptides' means that there is only one population of peptides being isolated [from the complex mixture]" (Id. at p. 14, second para., lines 6-8).

The Examiner's fourth contention is essentially correct. A central feature of Applicants' method is indeed the ability to selectively isolate, from a very complex mixture of mostly peptides

(both modified and unmodified) a desired target population of post-translationally modified peptides. Applicants have, therefore, presently amended independent claims 1 and 30 (as discussed with the Examiner) to clarify in the preamble and step (c) and step (d), respectively, of these claims that a "target" population of modified peptides desired to be isolated is in fact isolated by the invention.

The Examiner's third contention is also correct. Applicants have indeed chosen to claim their invention in terms that are commensurate in scope with the breadth of the disclosed invention. The complex mixture of peptides employed in Applicants' method need *not* consist *only* of peptides. No such limitation is disclosed in the specification. To the contrary, the complex mixture of peptides necessarily contains a diverse range of different small peptides (both modified and unmodified) *but may well also contain* other non-peptide cellular constituents, such as whole proteins, nucleic acids, and cell wall carbohydrates (however, in some preferred embodiments, the complex mixture might only contain peptides). The specification as filed makes this point clear. For example, the definitions of "complex mixture of peptide" and "proteinaceous preparation" on page 21 indicate that both peptide and protein may be present in the complex mixture. Similarly, there is an entire section (on pages 23 to 27) devoted to the nature of proteinaceous preparations and complex mixtures useful in the claimed method, which makes clear that the mixture can be a crude lysate containing many other cellular constituents (*see* p. 24, last paragraph) and need only be *significantly digested so as to be mostly peptides, as opposed to mostly proteins* (*see* p. 25, last paragraph).

The teachings of the specification on this point are entirely consistent with the goal of the invention, which is to provide a suitable and simple method for obtaining a "global snapshot" of a given type of post-translational modification in a given biological sample. In order to do that, one must examine many, if not all, *peptides* containing the desired modification, because, due to the fact that many proteins contain multiple modification sites, merely examining modification at the whole protein level will *not* provide the desired global snapshot.[1] Applicants' attorney emphasized in the December 15th telephonic interview that there is nothing in the specification that can be construed or relied on as/for limiting Applicants' invention as claimed to 100% peptide mixtures. In sum, it is not necessary to Applicants' invention that there be *no* proteins (or any other non-peptide components) in

[1] On this point, the Examiner is referred again to Raggiaschi *et al.* (Ref. DD, of record in this case), discussed at length in Applicants' prior Response, which teaches that in order to broadly identify phosphorylation sites it is necessary to isolate phospho-*peptides* (because merely isolating phospho-*proteins*, each of which may have multiple unique phosphorylation sites, is not adequate to identify the phospho-sites). *See* Raggiaschi at p. 35, first paragraph, and pgs. 34-41 generally.

the complex mixture from which the desired modified peptides will be isolated. The complex mixture may in fact contain such non-peptide components, although the skilled artisan will readily appreciate, from the detailed specification, that a substantially digested (*i.e.* mostly peptide as opposed to mostly protein) mixture is desired in order to accomplish the goal of the invention. Rather, what the invention requires is the ability to selectively isolate, from a very complex mixture of peptides, a desired target population of post-translationally modified peptides (*e.g.* isolating nitrosylated peptides from phosphorylated, acetylated, and methylated peptides, as well as from unmodified peptides, or isolating phosphotyrosine-containing peptides from phosphoserine- and phosphothreonine-containing peptides, as well as other undesired modified and unmodified peptides). This ability was an unsolved need in the art until the time of Applicants' invention.

It is this selective isolation element of the invention, explicitly required by claims 1 and 30, that is missing from Kanner, Wirth, and all other prior art. Accordingly, the Examiner's first and second contentions above are incorrect, as further discussed in the following Remarks.

I. ***The Maintained Novelty Rejections over Kanner and Wirth are Improper and Unsustainable Because Both References Fail to Teach Each and Every Element of the Invention as Claimed.***

The Examiner is reminded that a cited reference *only* anticipates a claimed invention if the reference discloses *each and every element or limitation* of the subject matter *as claimed*; the so-called "all elements" rule. *See* MPEP §706.02; MPEP §2131, *citing Verdegaal Bros. v. Union Oil of Cal.* (Fed. Cir. 1987). The Examiner *bears the burden* of establishing that a single reference in fact discloses each and every element, must consider the reference as whole, and may *not* read teachings into a reference that do not exist. MPEP §2131. The Kanner and Wirth references continue to fail to meet this test because (for the reasons already set out by Applicants' in their previous Responses) they do not teach *each and every* element or limitation of the claimed subject matter.

Independent claim 1 (as presently amended) reads as follows:

1. A method for *isolating a target population of naturally-occurring post-translationally modified peptides from a complex mixture of peptides*, said method comprising the steps of:

(a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises naturally-occurring post-translationally modified peptides from two or more different proteins;

(b) contacting said proteinaceous preparation with at least one immobilized post-translational modification-specific antibody; and

(c) *isolating said target population of naturally-occurring post-translationally modified peptides specifically bound* by said immobilized modification-specific antibody in step (b).

Independent claim 30 (as presently amended) reads as follows:

A method for *isolating a target population of phosphopeptides from a complex mixture of peptides*, said method comprising the steps of:

(a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises a complex mixture of peptides comprising phosphopeptides from two or more different proteins;

(b) fractionating phosphopeptides in said preparation by reversed-phase chromatography to produce a fractionated proteinaceous preparation;

(c) contacting said proteinaceous preparation with at least one immobilized motif-specific, context-independent antibody that binds a motif comprising at least one phosphorylated amino acid;

(d) *isolating said target population of phosphopeptides specifically bound* by said immobilized antibody in step (b); and

(e) characterizing said phosphopeptides isolated in step (d) by mass spectrometry (MS), tandem mass spectrometry (MS-MS), and/or MS^3 analysis.

The Examiner's attention is again drawn to the text italicized for emphasis: Both claims explicitly require, in their preamble and step (c) or step (d), respectively, that the claimed method *isolates a population of post-translationally modified peptides from a complex mixture of peptides.* Kanner and Wirth each completely fail to teach these required elements of the claimed method. Even *if* Kanner and Wirth are taken to describe using a complex mixture of peptides as presently alleged by the Examiner (which allegation itself is incorrect, as discussed below), the references still clearly fail to teach *isolating a desired target population of post-translationally modified peptides* as required by the claims.

The limited method disclosed in Kanner and Wirth can be summed up as being distinguished from the present invention in two important respects. First, the presently claimed method *starts* with a complex peptide mixture that is very different from that taught and employed in the cited references. Second, the presently claimed method *ends* with a different result (isolating and capturing desired modified peptides) from that taught and accomplished in the cited references (isolating proteins but losing peptides).

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Kanner & Wirth Fail to Employ a Complex Mixture of Peptides.

As noted above, the Examiner contends that it is *inherent* that the crude cell lysate employed in Kanner and Wirth also contains some peptide, and therefore such lysate may be considered a "complex mixture of peptides" as taught by the present invention. This contention is incorrect.

Kanner and Wirth both describe nothing more than employing *crude cell lysates* in their protein immunoprecipitation methods. An undigested crude cell lysate, which is well known in the art to largely comprise *whole protein* (along with a variety of other non-protein constituents, like nucleic acid, cell membrane carbohydrates, etc.) cannot fairly be considered a complex mixture of peptides within the scope of the present invention. It is well know to the skilled artisan that the normal biological state characterized by a crude cell lysate is one in which whole, functional proteins are present and acting in their biological capacity, not one in which such proteins have been highly fragmented into peptides (which fragmentation typically only occurs *in vivo* in protein catabolism and clearance mechanisms). Accordingly, the cell lysates described in Kanner and Wirth comprise mostly protein (*not* mostly peptide), as opposed to Applicants' method, which employs a complex mixture of peptides (described in the Specification as being *significantly digested so as to be mostly peptides, as opposed to mostly proteins* (*see* p. 25, last paragraph)). Applicants invention employs such a complex mixture of peptides because the goal of (and problem solved by) the invention to isolate most, if not all, of the post-translational protein modification sites of interest in a given sample so as to get a truly global snapshot of that modification in the sample.

The Examiner's attention is again drawn to the evidence in the record establishing that merely isolating the modified proteins, rather than the modified peptides derived from such protein, is insufficient for this purpose, since many proteins contain multiple modification sites that can only be detected by isolating all the peptides comprising those sites. *See* Raggiaschi at p. 35, first paragraph, and pgs. 34-41 generally (Ref. DD, of record). In contrast, the skilled artisan, reading Kanner and Wirth, would quickly appreciate that the cell lysate employed is mostly protein, and indeed *is intended to be*, because those whole proteins are then separated by molecular weight on a gel (which would be a pointless step if mostly peptides were present in their sample). In fact, Kanner and Wirth both expressly disclose *using a cocktail of proteolytic enzyme inhibitors specifically to prevent proteins being digested/fragmented into peptides.*

The Examiner has in fact, by virtue of arguing *inherent* disclosure, acknowledged that Kanner and Wirth fail to explicitly teach the complex mixture of peptides required by the claims. The

Examiner is reminded that, in order to rely on assertion of inherency, she must *clearly establish, with evidence* apart from those references, that the "missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of skill in the art." *See Continental Can Co. USA, Inc. v. Monsanto Co.*, 948 F.2d.1264 (Fed. Cir. 1991) (emphasis added); *see also* MPEP §2131.01 (III). The Examiner has presently not supported her allegation with *any* evidence. Moreover, such a showing is not possible because the complex mixture of peptides employed in the claimed invention is *not* necessarily present in a cell lysate such as that taught by Kanner and Wirth (as noted, peptides are largely not present due to their use of proteolytic enzyme inhibitors). Indeed, the Examiner herself has stated in the outstanding Office Action that such peptides are "possibly present" in the lysates of Kanner and Wirth (*See* November 20, 2006 Office Action at p. 14, first para., lines 12-13). The law is clear that "mere probabilities or possibilities" are *not* sufficient to establish inherency. *See Crown Operations, Intl. v. Solutia*, 289 F.3d 1367 (Fed. Cir. 2002). In short, the Examiner has failed to establish that the missing element, required by the claims, is inherent in the cited references.

The Examiner is again reminded that she bears the burden of establishing that Kanner teaches each and every element of the claimed subject matter: "The identical invention must be shown in as complete of detail as is contained in . . . the claim." *See* MPEP §2131, citing *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226 (Fed. Cir. 1989). Kanner fails to disclose, in identical detail, the invention of claims 1 and 30, because it fails to teach the use of complex mixture of peptides within the scope of the claims. Accordingly, the references therefore fail to anticipate the present invention on this ground alone.

Kanner & Wirth Fail to Teach Isolating a Target Population of Post-Translationally Modified Peptides.

As noted above, the Examiner contends that, because Kanner & Wirth inherently contain a complex mixture of peptides and employ a phospho-tyrosine antibody, the references must also inherently teach the selective isolation of a target population of modified peptides from that complex mixture, as taught by the present invention. This contention is incorrect.

Kanner and Wirth (as discussed at length in Applicants' previous paper (*see* p. 24-25 of August 24, 2006 Amendment and Response)) disclose no more than the immunoprecipitation of tyrosine-phosphorylated *proteins* from mixtures of *proteins* using well-known prior art techniques. Neither references relates in any way to the selective isolation of modified peptides from complex peptide mixtures, and both references utterly fail to provide any teaching on this required element of

Applicants' invention as claimed (*see* both preamble and step (c) and step (d), respectively, of independent claims 1 and 30).

Kanner, entitled "Immunoaffinity purification of tyrosine-phosphorylated cellular proteins," discloses that tyrosine-phosphorylated proteins in a cell extract may be immunoprecipitated using a phosphotyrosine-specific antibody, and then separated by gel electrophoresis prior to further analysis. Kanner utterly fails to disclose the selective isolation of a desired population of post-translationally modified peptides from all other populations of peptides (both modified and unmodified) that may exist in the sample. This selective isolation is a central and important feature of Applicants' claimed method, as recited in both the preamble and step (c) and step (d), respectively, of independent claims 1 and 30, and is simply not taught explicitly or inherently by Kanner. This is all the more true, because Kanner, when taken in its entirety, has nothing to do with isolating post-translationally modified peptides from complex peptide mixtures. Rather, the reference is only concerned with, and teaches no more than, the general isolation of phosphotyrosine-containing proteins by immunoprecipitation, which is for the purpose of then raising antibodies against those whole proteins. The paper is simply devoid of any teaching, or even suggestion, that anti-phosphotyrosine antibodies can be successfully employed in immunoaffinity format to selectively isolate a target population of phosphorylated peptides from a complex mixture of peptides.

In fact, even more telling, Kanner explicitly teaches separating the tyrosine-containing proteins, which have been immunoprecipitated, on a gel on the basis of their mass/size. Assuming that any peptides were even present in that immunoprecipitate, *those peptides would likely have run off the end of the gel and been completely lost during such gel resolution.* Accordingly, Kanner completely fails to in any way actually *isolate* a desired target population of modified peptides as required by the present claims. Kanner thus fails to teach an important required element of Applicants' claimed invention.

Wirth similarly is completely devoid of any teaching, or even suggestion, that a desired target population of post-translationally modified peptides can be immunoaffinity isolated from a complex mixture of peptides. Rather, Wirth merely describes (in Section 3.3, p. 121) using a phosphotyrosine antibody to immunoprecipitate tyrosine-phosphorylated proteins in a cell extract. Those precipitated proteins are *then* separated by 2-D gel, and can be excised and then partially sequenced as intact protein using the Edman method. Wirth utterly fails to disclose the selective isolation of a desired population of post-translationally modified peptides from all other populations of peptides (both modified and unmodified) that may exist in the sample. This selective isolation is a central and

important feature of Applicants' claimed method, as recited in both the preamble and step (c) and step (d), respectively, of independent claims 1 and 30, and is simply not taught explicitly or inherently by Wirth. This is all the more true, because Wirth, when taken in its entirety, has nothing to do with isolating post-translationally modified peptides from complex peptide mixtures. Rather, the reference is only concerned with, and teaches no more than, the general isolation of phosphotyrosine-containing proteins by immunoprecipitation, which is carried out for the purpose of building a database of known proteins in rat epithelial cells and their coordinates on a 2-dimensional gel map. The paper is simply devoid of any teaching, or even suggestion, that anti-phosphotyrosine antibodies can be successfully employed in immunoaffinity format to selectively isolate a target population of phosphorylated peptides from a complex mixture of peptides.

In fact, even more telling, Wirth (as with Kanner) explicitly teaches separating the tyrosine-containing proteins, which have been immunoprecipitated, on a gel on the basis of their mass/size. Assuming that any peptides were even present in that immunoprecipitate, *those peptides would likely have run off the end of the gel and been completely lost during such gel resolution.* Accordingly, Wirth completely fails to in any way actually *isolate* a desired target population of modified peptides as required by the present claims. Wirth thus fails to teach an important required element of Applicants' claimed invention.

The Examiner has in fact, by virtue of arguing *inherent* disclosure, acknowledged that the Kanner and Wirth fail to teach the selective isolation of a target population of post-translationally modified peptides (from a complex peptide mixture) required by the claims. The Examiner is reminded that, in order to rely on assertion of inherency, she must clearly establish, with *evidence* apart from those references, that the "missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of skill in the art." *See Continental Can Co. USA, Inc. v. Monsanto Co., supra.* (emphasis added); *see also* MPEP §2131.01 (III). The Examiner has presently not supported her allegation with *any* evidence. Moreover, such a showing is not possible because (i) the ability to selectively isolate a target population of phosphorylated peptides (*e.g.* phosphothreonine-containing peptides) is *not* necessarily present in the ability to immunoprecipitate phosphotyrosine-containing proteins as taught by Kanner & Wirth, (ii) the cited references themselves disclose a gel separation step in which any peptides actually present *would likely have been lost* instead of isolated (as discussed above), and (iii) the insufficiency of the Kanner and Wirth methods to selectively isolate modified peptides is irrefutably evidenced by the prior art of record in this case (*see* Section II below). Again, the law is clear that "mere probabilities

or possibilities" are not sufficient to establish inherency. *See Crown Operations, Intl. v. Solutia, supra.* In short, the Examiner has failed to establish that the missing element, required by the claims, is inherent in the cited reference.

The Examiner is again reminded that she bears the burden of establishing that Wirth teaches each and every element of the claimed subject matter: "The identical invention must be shown in as complete of detail as is contained in . . . the claim." *See* MPEP §2131, citing *Richardson v. Suzuki Motor Co., supra.* Both Kanner & Wirth fail to disclose, in identical detail, the invention of claims 1 and 30, because the references fail to teach the selective isolation of a desired target population of post-translationally modified peptides from a complex mixture of peptides, as required by the claims. Accordingly, the references therefore fail to anticipate the present invention on this ground alone.

Since Kanner and Wirth fail the bright-line requirement of teaching *each and every element and limitation of the claimed method*, the presently claimed subject matter is novel and patentable over these references (and all other prior art).[2] Accordingly, the maintained novelty rejection of claims 1, 2, 4, 5, 10, and 13-23 is improper as a matter of fact and law, and should be withdrawn.

II. ***The Maintained Novelty Rejections over Kanner and Wirth are Improper and Unsustainable Because Neither Kanner or Wirth is Enabling for the Claimed Method.***

The Examiner is reminded that a cited reference *only* anticipates a claimed invention if the reference is enabling for the subject matter *as claimed. See* MPEP §2121.01, *citing In re Hoeksema*, 158 U.S.P.Q. 596 (CCPA 1968). Stated another way, "A §102(b) reference must sufficiently describe the claimed invention to have placed the public in possession of it." *Paperless Accounting, Inc. v. Bay Area Rapid Transit Sys.*, 231 U.S.P.Q 649 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 933 (1987). The test for determining whether a prior art reference is enabled for a claimed invention is the same test for determining whether a specification is enabled: the cited reference must teach the invention in sufficient detail to enable one of skill in the art *to make and use it without undue experimentation. Minnesota Mining & Mfg. Co. v. Chemque, Inc.*, 64 U.S.P.Q.2d 1270 (Fed. Cir. 2002).

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[2] Furthermore, since these references fail to meet all elements of the broadest independent claims, they also fail to meet all elements of any of the dependent claims. In some instances, the Examiner has improperly rejected dependent claims with elements that are simply not taught at all in the cited references (for example, Wirth is devoid of any teachings relating to mass spectrometric identification of peptide sequence, despite the Examiner assertion to the contrary (*see* November 20, 2006 Office Action at p. 5).

Turning to the disclosures of Kanner and Wirth, not only do both references fail to teach each and every element of the claimed subject matter, but both references further fail to enable it. Both references utterly fail to provide *any teaching* (much less detailed teaching) on the conditions and steps necessary to successfully selectively immunoaffinity isolate a desired population of post-translationally modified peptides from a complex mixture of peptides. Rather, their disclosures are limited to describing how full-length *proteins* may be immunoprecipitated (and subsequently separated by tedious gel separation) from a mixture of proteins, which techniques are *not* suitable for isolating peptides in accordance with the claimed method. Given the limited teachings of Kanner and Wirth, a person of skill in this art would, at the time the present application was filed, have needed to resort to *extensive and undue* experimentation to attempt to selectively isolate a target population of modified *peptides* using the protein immunoprecipitation method disclosed by the cited references.

Indeed, the best evidence of this undue experimentation and the fact that Kanner and Wirth are not enabling is:

1. The disclosures of Kanner and Wirth themselves, which describe and employ a gel separation step in which any peptides even present in the immunoprecipitate *would likely have been lost by running off the end of the gel* instead of being isolated; and

2. The state-of-the-art publications of record in this case that firmly establish *the failed prior attempts* to accomplish what Applicants' method accomplished for the first time, and which clearly state that prior art protein immunoprecipitation methods (as taught by the cited references) *don't work* for selectively isolating modified *peptides*. *See* Refs. CF, CG, CZ and DA-DD, all of record, discussed at length in Applicants' previous Responses and Interviews.

A person of skill in the art, following the teachings of Kanner and Wirth, would firstly have to resort to extreme and undue experimentation to attempt to divine the conditions and parameters suitable for selectively isolating desired modified peptides (according to Applicants' invention)[3] from papers that relate only to immunoprecipitating proteins. Secondly, such artisan would in fact *not have succeeded* in isolating phosphotyrosine-containing peptides from the immunoprecipitate of Kanner and Wirth because those peptides, if even present, would likely have been entirely lost by employing the gel separation step of those cited methods. The Examiner's insistence that Kanner and Wirth are enabling for Applicants' claimed invention is not supported by *any* evidence and is

directly refuted by the disclosures of Kanner and Worth, as well as the cited art of record in this case.

For example, Mann (Ref. CG) expressly states the prevailing view (at the time the present application was filed) that phospho-specific antibodies were *not suitable* for selectively isolating phosphorylated *peptides* from mixtures, due to various technical limitations. *See* Mann p. 261, last paragraph. Marcus (Ref. CF) expressly concludes that the detection of phosphorylation sites (phosphopeptides) using such phospho-specific antibodies is "almost impossible" due to various technical limitations, including binding affinity. *See Marcus* p. 2635, end of 3.2.1. Quadroni (Ref. DA) expressly states that "As a general rule, all these antibodies behave quite poorly as affinity reagents, especially bad towards small peptides, and their main application remains in Western blotting." *See* Quadroni at p. 201, end of 1.3. The paper also expressly states *that attempts to use anti-phosphotyrosine antibodies to isolate phosphotyrosine-containing peptides had failed.* The other references of record underscore the same point. The technical limitations referred to by these prior art references were the very ones overcome by Applicants in developing their novel invention. Indeed, the Examiner's attention has previously been directed as well to Conrads (Ref. CZ), a review article of Applicants' method that appeared in the leading journal, *Nature Biotechnology*, shortly after Applicants published an article about their method (following the filing of this application), and further evidences the novelty of Applicants' method. In the review, the authors expressly conclude that the Applicants' invention "address[es] the deficiency" in prior art proteomics approaches for isolating phosphopeptides, and go on to highlight several of the problems (*e.g.* low abundance of phosphopeptides from complex mixtures, need for enrichment, etc.) with prior art approaches that remained unsolved until the Applicants' invention. *See Conrads* at p. 36, end of first para.

Similarly, Reinders *et al.*, (Ref. DB) expressly states the prevailing view that while phospho-specific antibodies have historically been used to immunoprecipitate phosphorylated *proteins* (as taught by Kanner and Wirth) these antibodies and approaches have *not been suitable* for selectively isolating phosphorylated *peptides* from mixtures, and *other methods have to be applied. See* p. 4054, first paragraph. Peters *et al.*, (Ref. DC) also represents and expressly states the prevailing view that phospho-specific antibodies have been used to immunoprecipitate phosphorylated *proteins* (which then must typically be separated on a 2-gel before analysis), and that these antibodies and approaches have *not been suitable* for selectively isolating phosphorylated *peptides* from mixtures, due to various technical difficulties. *See* p. 314, second and third paragraphs.

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[3] Which conditions and technical parameters, as developed by Applicants', are described in detail in their 133-page specification as filed.

The Examiner is reminded that, "A §102(b) reference must sufficiently describe the claimed invention to have placed the public in possession of it." *Paperless Accounting, Inc. v. Bay Area Rapid Transit Sys., supra.* The Examiner's contention that a skilled artisan somehow possessed Applicants' invention, based on the disclosures of Kanner and Wirth, despite that those references themselves failed to accomplish what Applicants' invention accomplishes and that such failure and insufficiency is clearly and irrefutably evidenced by the art of record, is improper and unsupportable.

Accordingly, Kanner and Wirth both fail to anticipate the presently claimed method because these references fail to enable it. The novelty rejection of claims 1, 2, 4, 5, 10, and 13-23 is therefore improper and should be withdrawn.

OBVIOUSNESS REJECTIONS (MAINTAINED) -- BASED ON WIRTH, LITTLE, PIDGEON & STOUGHTON

The Examiner has maintained the rejection of claims 3, 6-9, 11, 24-28, 30-39, and 49-52 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*Electrophoresis* (1993), *supra.*) in view of Little *et al.* (U.S. Pat. No. 6,322,970, (issued Nov. 27, 2001) (hereinafter "Little") – already of record in this case). The Examiner asserts that while Wirth fails to disclose certain additional aspects of preferred embodiments of Applicants' method (which are recited in the rejected dependent claims), Little teaches or suggests these steps, hence the claimed subject matter is obvious.

The Examiner has also maintained the rejection of claim 12 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*supra.*) in view of Little *et al.* (*supra.*) and in further view of Pidgeon *et al.* (U.S. Pat. No. 6,579,720, (issued June 17, 2003) (hereinafter "Pidgeon") – already of record in this case). The Examiner asserts that while Wirth fails to disclose the preferred embodiments of Applicants' method (which are recited in rejected dependent claim 12 and claim 11), Little combined with Pidgeon teach or suggest these steps, hence the claimed subject matter is obvious.

Lastly, the Examiner has also maintained the rejection of claim 29 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*supra.*) in view of Little *et al.* (*supra.*) and in further view of Stoughton *et al.* (U.S. Pat. No. 5,965,352, (issued October 12, 1999) (hereinafter "Stoughton") – already of record in this case). The Examiner asserts that while Wirth and Little fail to disclose the preferred embodiments of Applicants' method (which are recited in rejected dependent claim 29), Wirth and Little combined with Stoughton teach or suggest these steps, hence the claimed subject matter is obvious.

Applicants respectfully disagree and, for the same reasons set out at length in Applicants' previous Response (filed August 24, 2006), submit that the Examiner *has still failed to establish the required prima facie showing of obviousness*. Further, the deficiencies of the primary reference, Wirth (discussed at length in Section I above), are in no way cured by the limited teachings of Little, Pidgeon, and/or Stoughton.

I. *The Maintained Obviousness Rejections are Improper and Unsustainable Because the Examiner has Failed to Establish the Required Prima Facie Showing.*

The Examiner is reminded that patent law mandates that she must establish *a prima facie* case of obviousness by establishing three elements: (i) that there is some suggestion or motivation in the references themselves – or if not, then in the knowledge generally available to those of skill in the art – to combine the teachings of the references; (ii) that there is some reasonable expectation of success, as evidenced by the cited references and/or other prior art, in so combining the teachings, and (iii) that the cited references teach or suggest each and every limitation of the claimed subject matter. *See* MPEP §§2142, 2143, *citing, e.g. In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). The mere fact that references can be combined is *not* sufficient to establish desirability or motivation to do so (*see* MPEP §2143.01, citing *In re Mills*, 916 F.2d 680) (Fed. Cir. 1990)).

In the present case, the Examiner has again failed to establish the required *prima facie* showing of obviousness because none of the three required elements has been met. As discussed at length in Section I above, the primary reference, Wirth, completely fails to teach, suggest, or make obvious Applicants' invention as presently claimed. The limited teachings of Wirth, and how the phosphoprotein immunoprecipitation method it discloses is distinguished from the present invention, have been described in detail above. Not only is there no teaching or suggestion of Applicants' method in Wirth, but a skilled artisan would have had absolutely *no motivation or expectation* that its teachings could be applied to successfully isolate modified *peptides* from complex mixtures of *peptides*. In fact, such artisan would have had an *expectation of failure* given the clear prior art failures and teaching away evidenced by the references of record in this case (Refs. CF, CG, CZ and DA-DD, discussed above).

Since the primary reference, Wirth, fails to render obvious the invention as most broadly claimed in independent claims 1 and 30, the claim rejections based on combinations of Wirth and the secondary references Little, Pidgeon, & Stoughton similarly fail. Accordingly, the subject matter of claims 3, 6-9, 11, 12, and 24-28 (as well as all other pending claims) is non-obvious and patentable over Wirth and the cited secondary references, and the rejection of these claims should be withdrawn.

Further, none of the secondary references – Little, Pidgeon, or Stoughton (nor any other references cited by the Examiner in this case) – taken alone or combined with Wirth, teach, suggest, or make obvious the presently claimed methods for *selectively isolating, from a complex mixture of peptides, a target population of naturally-occurring post-translationally modified peptides* by immunoaffinity isolation using a post-translational modification-specific antibody. The limited teachings and shortcomings of both of these references were extensively discussed and distinguished by Applicants previously (both in their in-person interviews and in previous Responses).

II. ***The Maintained Obviousness Rejections are Improper and Unsustainable Because the Secondary Evidence of Record Supports the Non-Obviousness of the Invention.***

The non-obviousness of the present invention over the prior art is irrefutably evidenced by references like Mann, Marcus, Quadroni, Conrads, and others (*see* Refs. CF, CG, CZ and DA-DD (discussed at length above)), all of which *teach away* from the invention *and evidence the failed prior art attempts* to accomplish what Applicants' have accomplished. The Examiner is reminded, "That the inventor achiev[ing] the claimed invention by doing what those skilled in the art suggested should not be done is a fact strongly probative of non-obviousness." *See Kloster Speedsteel AB v. Crucible, Inc.*, 793 F.2d 1565 (Fed. Cir. 1986) (emphasis added); *see also Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720 (Fed. Cir. 1990) (discussing prior publications which discourage the skilled artisan from doing what the inventor did evidence non-obviousness).

The novelty and non-obviousness of Applicants' method is further evidenced by the surprising results, long-felt but unsolved need, and commercial success associated with the invention, as discussed during the first and second in-person interviews by Dr. John Rush and in Applicants' previous Responses. Indeed, Conrads *et al.* (Ref. CZ, of record), clearly evidences the long-felt need filled by Applicants' invention by stating that the invention "address[es] the deficiency" in prior art proteomics approaches for isolating phosphopeptides. *See Conrads* p. 36, end of first para. The Examiner is reminded that such factors "must be given due weight by the Examiner" (*see In re Sernaker*, 702 F.2d 989 (Fed. Cir. 1983) (emphasis added)); *see also Custom Accessories Inc. v. Jeffrey-Allan Industries, Inc.*, 807 F.2d 955 (Fed. Cir. 1986) ("Under *Graham*, objective evidence of nonobviousness includes commercial success, long felt but unsolved need, failure of others, and copying. When present, such objective evidence must be considered") (emphasis added).

Here, the Examiner has not only failed to make the required *prima facie* showing of obviousness, but even if she had, that showing would be refuted by the secondary factors and evidence of record that strongly weigh in favor of the non-obviousness of Applicants' invention.

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Accordingly, the subject matter of claims 3, 6-9, 11, 24-28, 30-39, and 49-52 (as well as all other pending claims) is non-obvious and patentable over Wirth, Little, Pidgeon, & Stoughton (and all other cited references), whether taken alone or together, and the rejection of these claims should be withdrawn.

DOUBLE-PATENTING (STATUTORY) REJECTIONS

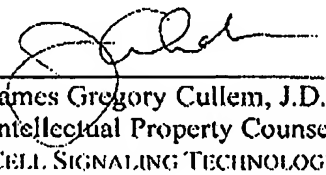
The Examiner has again provisionally rejected claims 1-29 under 35 U.S.C. §101 for "statutory" double patenting, as allegedly claiming the same invention as that of claims 1-29 of co-pending application USSN 10/175,486 (Rush *et al.* -- also owned BY CELL SIGNALING TECHNOLOGY, INC., the assignee of the present application).

Since the rejection is *provisional*, Applicants respectfully renew their request that this rejection be held in abeyance until such time as the present application or cited co-pending application issues as a patent, at which time Applicants will cancel or amend any identical claims in the remaining application.

Conclusion

For the reasons set forth above, the outstanding maintained novelty and obviousness rejections of the claims are improper and unsustainable, and should be withdrawn. The present claims are patentable and are in condition for immediate allowance. Withdrawal of the outstanding rejections is respectfully requested, and prompt allowance and issuance of the claims is earnestly solicited. Similar issuance and allowance of the related continuation-in-part case (USSN 10/175,486)(Atty. Docket No. CST-201) is also earnestly solicited, as the issues raised in the remaining rejections in that case are the same as those discussed in this paper. If there are any questions regarding these amendments and remarks, the Examiner and her SPE are requested to call the undersigned attorney at the telephone number provided prior to the issuance of any further actions.

Respectfully submitted,



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